CLAIMS

What is claimed is:

1. A method comprising:

contacting a preparation of a recombinant protein that has been produced by mammalian cells with a reduction/oxidation coupling reagent, at a pH of about 7 to about 11, and isolating a fraction of the preparation of the recombinant protein with a desired conformation.

2. The method of claim 1 wherein the recombinant protein contains at least two domains.

3. The method of claim 2 wherein at least one domain of the protein has a stable conformation, and at least one domain of the protein has an unstable conformation.

4. The method of claim 1 wherein the recombinant protein comprises an extracellular domain of a receptor.

5. The method of claim 1 wherein the recombinant protein is a soluble form of a TNF-receptor.

6. The method of claim 1 wherein the recombinant protein is a Fc fusion protein.

7. The method of claim 6 wherein the preparation of the recombinant protein has been purified from a Protein A or Protein G column.

8. The method of claim 1 wherein the recombinant protein is selected from the group consisting of a soluble IL-4 receptor, a soluble IL-1 type II receptor, a soluble Flt3 ligand, a soluble CD40 ligand, CD39, CD30, CD27, a TEK/Ork, IL-15, a soluble IL-15 receptor, Ox 40, GM-CSF, RANKL, RANK, TRAIL, a soluble TRAIL receptor, tissue plasminogen activator, Factor VIII, Factor IX, apolipoprotein E, apolipoprotein A-I, an IL-2 receptor, an IL-2 antagonist, alpha-1 antitrypsin, calcitonin, growth hormone, insulin, insulinotropin, an insulin-like growth factor, parathyroid hormone, an interferon, superoxide dismutase, glucagon, an erythropoeitin, an antibody, glucocerebrosidase, an Fc-fusion protein, a globin, a nerve growth factor, an interleukin, and a colony stimulating factor.

9. The method of claim 1 wherein the pH is from about 7 to about 10.

10. The method of claim 9 wherein the pH is about 7.6 to about 9.6.

11. The method of claim 10, wherein the pH is about 8.6.

12. The method of claim 1 wherein the reduction/oxidation coupling reagent comprises glutathione.

13. The method of claim 12 wherein the ratio of reduced glutathione to oxidized glutathione is about 1:1 to about 100:1.

14. The method of claim 1 wherein the reduction/oxidation coupling reagent comprises cysteine.

15. The method of claim 1 wherein the contacting step is performed for about 4 to about 16 hours.

16. The method of claim 1 wherein the contacting step is performed at about 25°C.

17. The method of claim 1 wherein the contacting step is performed at about 4°C.

18. The method of claim 1 wherein the contacting step is quenched by acidification.

19. The method of claim 1 wherein the isolating step comprises one or more chromatography steps.

20. The method of claim 1 wherein the protein concentration is from about 0.5 to about 10 mg/ml.

21. The method of claim 1 wherein the ratio of reducing thiols in the reduction/oxidation coupling reagent to disulfide bonds in the protein is about 320:1 to about 64,000:1 (reducing thiols: disulfide bond).

22. The method of claim 1 further comprising formulating the fraction of the preparation of the recombinant protein with the desired conformation in a sterile bulk form.

23. The method of claim 1 further comprising formulating the fraction of the preparation of the recombinant protein with the desired conformation in a sterile unit dose form.

24. The method of claim 4 wherein the desired conformation has a higher binding affinity for a cognate ligand of the receptor.

25. The method of claim 5 wherein the desired conformation has a higher binding affinity for TNF.

26. The method of claim 25 wherein the TNF is TNF-alpha.

27. A method of promoting a desired conformation of a glycosylated recombinant protein, the method comprising

contacting a preparation of the glycosylated recombinant protein that contains a mixture of at least two configurational isomers of the glycosylated recombinant protein with a reduction/oxidation coupling reagent for a time sufficient to increase the relative proportion of the desired configurational isomer and

determining the relative proportion of the desired configurational isomer in the mixture.

28. The method of claim 27 wherein the glycosylated recombinant protein contains at least two domains.

29. The method of claim 28 wherein at least one domain of the glycosylated recombinant protein has a stable conformation, and at least one domain of the glycosylated recombinant protein has an unstable conformation.

30. The method of claim 27 wherein the glycosylated recombinant protein comprises an extracellular domain of a receptor.

31. The method of claim 27 wherein the glycosylated recombinant protein is a soluble form of a TNF-receptor.

32. The method of claim 27 wherein the glycosylated recombinant protein is a Fc fusion protein.

33. The method of claim 32 wherein the preparation of the glycosylated recombinant protein has been purified from a Protein A or Protein G column.

34. The method of claim 27 wherein the glycosylated recombinant protein is selected from the group consisting of a soluble IL-4 receptor, a soluble IL-1 type II receptor, a soluble Flt3 ligand, a soluble CD40 ligand, CD39, CD30, CD27, a TEK/Ork, IL-15, a soluble IL-15 receptor, Ox 40, GM-CSF, RANKL, RANK, TRAIL, a soluble TRAIL receptor, tissue plasminogen activator, Factor VIII, Factor IX, apolipoprotein E, apolipoprotein A-I, an IL-2 receptor, an IL-2 antagonist, alpha-1 antitrypsin, calcitonin, growth hormone, insulin, insulinotropin, an insulin-like growth factor, parathyroid hormone, an interferon, superoxide dismutase, glucagon, an erythropoeitin, an antibody, glucocerebrosidase, an Fc-fusion protein, a globin, a nerve growth factor, an interleukin, and a colony stimulating factor.

35. The method of claim 27 wherein the pH is from about 7 to about 10.

36. The method of claim 35 wherein the pH is about 8.6.

37. The method of claim 27 wherein the reduction/oxidation coupling reagent is selected from the group consisting of glutathione, cysteine, DTT (dithiothreitol), 2-mercaptoethanol and dithionitrobenzoate.

38. The method of claim 37 wherein the reduction/oxidation coupling reagent comprises reduced glutathione.

39. The method of claim 38 wherein the reduced glutathione is at a concentration of about 1 mM to about 10 mM.

40. The method of claim 37 wherein the reduction/oxidation coupling reagent comprises reduced cysteine.

41. The method of claim 37 wherein the ratio of reducing thiols in the reduction/oxidation coupling reagent to disulfide bonds in the protein is about 320:1 to about 64,000:1 (reducing thiols: disulfide bond).

42. The method of claim 27 wherein the protein concentration is from about 0.5 to about 10 mg/ml.

43. The method of claim 27 wherein the contacting step is performed for about 4 to about 16 hours.

44. The method of claim 27 wherein the contacting step is performed at about 25°C.

45. The method of claim 27 wherein the contacting step is performed at about 4°C.

46. The method of claim 27 wherein the contacting step is quenched by acidification.

47. The method of claim 27 wherein the determining step comprises one or more chromatography steps.

48. The method of claim 27 wherein the determining step comprises a binding reaction.

49. The method of claim 27 comprising isolating a fraction of the preparation of the glycosylated recombinant protein with the desired configurational isomer.

50. The method of claim 49 comprising formulating the desired configurational isomer in a sterile unit dose form.

51. The method of claim 30 wherein the desired configurational isomer has a higher binding affinity for a cognate ligand of the receptor.

52. The method of claim 31 wherein the desired configurational isomer has a higher binding affinity for TNF.

53. The method of claim 52 wherein the TNF is TNF-alpha.

54. A method comprising formulating into sterile unit dose form a recombinant protein that has been produced by mammalian cells, contacted with a reduction/oxidation coupling reagent, and isolated from the fraction of the protein with an undesired conformation.

55. A pharmaceutical composition of a TNFR:Fc produced by the method of claim 54.